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13. ABSTRACT (Maximum 200 Words)

The CDK inhibitor $p27^{Kip1}$ acts as a negative regulator in the cell cycle and in prostate tumorigenesis. Either the loss of p27 or over-expression of its key regulator ubiquitin E3 ligase SKP2 is associated with prostate cancer. Prostate-sepcific expression of SKP2 is sufficient to induce hyperplasia, displasia, and low grade carcinoma in the mouse prostate gland. We propose to examine whether SKP2 cooperates with or is regulated by tumor suppressors such as Pten or Nkx3.1 implicated in prostatic tumorigenesis. In the past year, we have initiated the experiments by breeding and expansion of the colonies, crossing the SKP2 transgenic mice into Pten +/- strains, and ordering Nkx3.1 mice. Compound mice of SKP2 transgene and Pten+/- were made. Our initial studies suggest that there is no substantial difference in tumor frequency and grade in the SKP2 and Pten +/- compound mice versus single genetic alterations. These results suggest that PTEN may act through SKP2 for tumorigenesis. Since most of Pten null mice died early due to tumor growth in other tissues, we are in the process using Pten conditional knockout mice and prostate-specific-Cre recombinase to remove Pten in the prostate gland to determine the relationship between PTEN null and SKP2 expression.

14. SUBJECT TERMS

$P27^{Kip1}$, SKP2, PTEN, CDK, SCF^{SKP2}, Tg-SKP2

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Introduction:

Low or absent expression of the CDK inhibitor $p27^{Kip1}$ is closely associated with malignant prostate carcinomas or other cancers with poor prognosis [1-6]. P27 is primarily regulated by ubiquitin-dependent proteolysis in the cell cycle [2]. We have shown that the ubiquitin-dependent degradation of p27 is mediated by the SCF^{SKP2} ubiquitin E3 ligase in the cell cycle [7-11]. In both premalignant lesions of prostatic intraepithelial neoplasm and prostate cancer, the level of SKP2, the substrate-specificity subunit of SCF^{SKP2}, is frequently elevated that is inversely correlated with low levels of p27 [12-14]. The elevated expression of SKP2 may thus be the primary cause for p27 downregulation in prostate cancer. To examine the role of SKP2 in prostate tumorigenesis, we have established SKP2 transgenic mouse lines that specifically express SKP2 in the prostate gland. These studies suggest that SKP2 is an oncoprotein in the mouse prostate and its expression is sufficient to induce the p27 downregulation and to cause a wide spectrum of hyperplasia, dysplasia, and low grade carcinoma in the prostatic epithelium [11]. In human prostate cancers, elevation of SKP2 is also inversely correlated with the loss of tumor suppressor PTEN [12,15]. We propose that SKP2 is a major cell cycle regulator in the prostate epithelium and is rate limiting during prostatic tumorigenesis. The SKP2 pathway may either cooperate with or be regulated by the other major tumor suppressor pathways, such as the loss of PTEN or Nkx3.1, that have been implicated in prostate tumorigenesis [16-18]. Because of their critical role in prostate cancer, the SKP2/p27 pathway may be a critical target of many anti-cancer drugs or dietary agents including vitamin D3 [19-21]. Alteration of SKP2 expression may affect the efficacy of these chemicals. Our specific aims are: 1) To determine whether SKP2 collaborates with other proliferation processes by transient expression in the prostatic epithelium. 2)To examine whether SKP2 cooperates with Pten deficiencies to promote prostatic tumorigenesis. 3) To determine whether the SKP2/p27 pathway cooperates or overlaps with the loss of Nkx3.1 for prostatic neoplasm in mouse. 4) To evaluate the relationship between SKP2 expression and the sensitivities of prostatic preneoplasm/neoplasm towards anti-cancer drugs such as vitamin D3.

Body:

In the past year, we have started our investigation based on our proposal. In general, we have started breeding colonies to increase the colony size. We also making and/or obtaining reagents or mouse lines that are necessary for our experiments. Several progress are made: for aim1, we have started to amplify the SKP2 adenovirus and Lac Z control adenovirus in 293 cells, and titrate their titers. The expression of SKP2 was examined using prostate cancer cell lines using western blot analysis to examine both the expression of SKP2 and its effect on p27 levels. Since the titer of the virus was not very high, we will continue to amplify the viruses and concentrate the viruses using CsCl gradient ultra-centrifugation. These viruses will be used to examine on the effect of SKP2 expression on proliferation of prostate epithelium as proposed in the aim 1.

For aim 2, we have increased the colony sizes of two mouse lines of SKP2, 9079 and 9033, and a PTEN +/- mouse strain obtained from Dr. Hong Wu, UCLA, by breeding. These two SKP2 mouse strains were subsequently crossed into the PTEN +/- mice. Several rounds of breeding were conducted because the original breeders are about one year old and thus produced less progenies. The SKP2 transgenic and Pten heterozygotes (+/-) compound mice were obtained after genotyping. They were examined for the potential effect of Pten deficiency on the progression of prostate tumors in SKP2 mice or verse versa. They were compared with the prostate glands isolated from the control and SKP2 alone or Pten+/- alone mice at 3, 6, and 7 months after birth.

Histological analysis with H&E and SKP2 were used for these examinations. While the prostate glands isolated from SKP2 transgenic mice and PTEN +/- mice displayed substantial hyperplasia and displasia in about 3-6 months, our initial study did not reveal any significant effect of Pten +/- in the SKP2 background in the compound mice on both the tumor grade, fraction, and onset time within the time frame we have examined. Since the cooperativity between PTEN and Nkx3.1 has been reported to occur relatively late in advanced age [18], we are to examine the effect of Pten deficiency in SKP2 transgenic mice at later time points, 9-18 months, or longer if necessary. We are also examining the effect of Pten loss in the endogenous SKP2 and p27 levels and distribution. Based on several of our previous studies in cultured cells, the expression of SKP2 is negatively regulated by PTEN [9,22]. It is possible that one effect of Pten deficiency may cause the increase of SKP2 expression and the induction of endogenous SKP2 may be sufficient to induce hyperplasia and dysplasia in the mouse prostate gland, as we have observed in SKP2 transgenic mice [11]. We would like to test more on the regulation of SKP2 by PTEN in the prostate gland and will also examine the effect of Pten null mice on SKP2-induced prostate tumors. However, so far we have obtained very few Pten null mice (Pten -/-) and most of them died or sick that raises human concern so that we have to euthenize them within 3-4 months after birth. In general Pten+/- mice have been reported to have lower fertility rate and Pten null mice die because of malignancies in other tissues such as lymphomas or endometrium tumors [23]. To overcome these effect, we have obtained the Pten conditional knockout mice (Pten cnd) from Jackson laboratory and is in the process obtaining the prostate specific Cre recombinase under the probasin-promoter control from MMHCC, NCI. We will cross the SKP2 into the Pten conditional knockout mice and use the prostate-specific Cre recombinase (mouse strain: PB-CRE4) to knockout Pten in the prostate gland and examine the effect of Pten loss on SKP2-inudced prostate tumorigenic process.

For aim 3, we have ordered Nks3.1 +/- mice from MMHCC, NCI. However, MMHCC no longer carries the live animals of this strain and only has the frozen embryos. We are on the waiting list to obtain this mouse strain from MMHCC, NCI. We will also try to find other sources for this mouse strain.

For aim 4, we have started the colony breeding. However, because these experiments require substantial number of SKP2 mice and we are in the process of conducting this set of experiments.

Key research accomplishments:

In preparation: Loss of Pten may act through SKP2 for prostate tumorigenesis.

Reportable outcome:

None

Conclusion:

our initial study using limited number of mice suggested that there is no substantial difference in SKP2 induced prostatic tumors in either wild-type or Pten +/- background within 3-7 months after birth. One possibility is that Pten +/- may act through SKP2 for inducing prostate tumors. However, we will conduct more experiments to provide statistical value and also to examine longer time points for effect of these compound mice in prostate tumorigenesis. In addition, since Pten null mice are difficult to obtain due to high rate of tumors in other organs, we will use Pten conditional knockout mice to determine the loss of Pten on SKP2-induced tumors or verse versa. We will continue to

conduct other experiments as proposed in the grant by preparing SKP2 reagents and increase mouse colony sizes.

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